Introduction

Many epidemiological surveys of diet have analyzed the relation between fruit intake and lifestyle-related disease \(^1\). It has been claimed that fruit consumption reduces blood pressure \(^2\), hypertension risk \(^3\) and the prevalence of cerebral apoplexy \(^4\); prevents Alzheimer’s disease \(^5\) and gastric cancer \(^6,7\); improves oral function, i.e., teeth and gum condition in elderly persons \(^8\), glucose tolerance in middle-aged and older women \(^9\), and mental outlook in middle-aged and older men and women \(^10\). However, none of these reports clearly tested the protective efficacy of fruit consumption in an interventional study design.

Excess fruit consumption may lead to excess calorie intake and, in consequence, cause obesity and hyperuricemia \(^11\). It has also been reported that female college athletes increase fruit intake and decrease confectionery intake following dietary instruction \(^16\).

Grapefruit \((GF)\) \((Citrus X paradisi)\) contains 38 kcal, 89 g water, 9.6 g sugar, 1 g citric acid, 140 mg potassium, 36 mg vitamin C and 9 mg magnesium \(^17\) per 100 g fruit. Other components include limonene, furanocoumarins and flavonoids.

Previous studies identified the potential of GF to favorably affect metabolic syndrome \(^18\) and lipid metabolism \(^19\). The present study measured the change of the blood glucose, triglyceride and insulin following GF consumption by human volunteers, in combination with other foods.
Methods

Objective

Twelve healthy, non-obese, non-smoking women, (mean age 40.5 ± 4.2 years, BMI 22.0 ± 0.9) were chosen from 249 applicants who responded to an Internet advertisement in a social network service (Marsh Research, Setagaya-ku, Tokyo). Pregnant and lactating women, persons on medication or with high blood pressure, diabetes or a medical history of digestive surgery were excluded. Tests for each participant were conducted over five consecutive days between December 2010 and January 2011.

Prior written informed consent was obtained from all subjects. The aims and methods of the study were explained at meetings conducted at the Kikunodai Medical Clinic (Chofu-shi, Tokyo).

Fruit samples

GF were provided by Florida Department of Citrus (United States of America) via Amano and Associates (Minato-ku, Tokyo). The mean weight of GF used in the trial was 421 ± 2 g. Bread was provided by Shikishima Bread-Making Inc. (Nagoya-city, Aichi) and fried vegetables was provided by Seiyu (Kitaka-ku, Tokyo).

Trial design

<table>
<thead>
<tr>
<th>Day</th>
<th>Food intake</th>
<th>Energy content (kcal)</th>
<th>Carbohydrate (g)</th>
<th>Lipid (g)</th>
<th>Protein (g)</th>
<th>vitamin C (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>①</td>
<td>12/20 GF</td>
<td>81.9</td>
<td>20.7</td>
<td>0.2</td>
<td>1.9</td>
<td>77</td>
</tr>
<tr>
<td>②</td>
<td>12/21 Bread</td>
<td>158.0</td>
<td>29.8</td>
<td>2.2</td>
<td>4.8</td>
<td>0</td>
</tr>
<tr>
<td>③</td>
<td>12/22 GF + bread</td>
<td>237.1</td>
<td>49.8</td>
<td>2.4</td>
<td>6.7</td>
<td>77</td>
</tr>
<tr>
<td>④</td>
<td>12/24 GF + fried vegetables</td>
<td>297.4</td>
<td>36.0</td>
<td>16.0</td>
<td>5.1</td>
<td>80</td>
</tr>
<tr>
<td>⑤</td>
<td>1/5 Fried vegetables</td>
<td>202.5</td>
<td>14.6</td>
<td>14.8</td>
<td>2.7</td>
<td>3</td>
</tr>
</tbody>
</table>

Each subject was given 15 minutes to ingest the breakfast menu specified above. The breakfast start-time for each subject was staggered (5-minute intervals) to allow time for blood sampling, resulting in a 60-minute difference in the start and end time of the first and the last subject. On each sample day, blood samples were taken from each subject at 0, 30, 60, 120 and 180 minutes after the meal, and glucose and immunoreactive insulin (IRI) were measured. In addition, vitamin C was measured at 0 and 180 minutes on day-1, and glucose and triglyceride AUC was lower when GF was eaten prior to fried vegetables intake of fried vegetables without GF (Fig. 1c; p < 0.001).

The mean concentration of serum vitamin C increased from 9.67 ± 2.01 μg/ml prior to GF ingestion to 12.66 ± 2.35 μg/ml (+30.9%, p < 0.001) after 180 minutes (Fig. 2).

Insulin levels rose after meal ingestion, but the IRI 30 minutes after GF intake was lower than that after intake of bread or fried vegetables ingested without GF (Fig. 3a; p = 0.017). The AUC for IRI after GF intake was lower than that for all other menus (Fig. 3k; p < 0.05). The AUC of IRI/carbohydrate intake was lower when the FV was taken with GF than that after intake of fried vegetables without GF (Fig. 3c; p < 0.001).

Triglyceride level gradually increased 30, 60 and 120 minutes after fried vegetables intake (Fig. 4a), and the triglyceride AUC was lower when GF was eaten prior to fried vegetables (Fig. 4b; p = 0.049). Triglyceride AUC/carbohydrate intake was lower when GF was eaten prior to fried vegetables (Fig. 4c; p < 0.001).

Ethical considerations and conflict of interest

The study was completed at the Kikunodai Medical Clinic in compliance with the ethical principles of the Declaration of Helsinki, the Personal Information Protection Law, and with reference to the “Ordinance regarding the Good Clinical Practice” (Ministry of Health and Welfare Ordinance No. 28, March 27, 1997). The study was approved by the third-party ethical committee of the Fukushima Health Care Center (Osaka-city, Osaka) and the ethical committee of Doshisha University (approval number #0832). We declare there is no financial or other conflict of interest in the writing or publication of this paper.

Data analysis

Statistics were calculated with SPSS (IBM Japan, Minato-ku, Tokyo). Differences before and after the trial period were tested by paired-t (parametric) and Wilcoxon signed rank (non-parametric) tests. Group means were compared by Dunnnett’s test. The results are presented as mean ± standard deviation and p < 0.05 was assumed to be significant. The safety of the test product was evaluated by prevalence of adverse effects in individual subjects.

Result

In all diets, plasma glucose level rose 30 minutes after a test meal had been ingested (Fig. 1a), but glucose AUC did not differ between groups (Fig. 1b). In contrast, glucose AUC/carbohydrate intake for GF + bread and GF + fried vegetables was less than that for bread or fried vegetables ingested without GF (Fig. 1c; p < 0.001).

The mean concentration of serum vitamin C increased from 9.67 ± 2.01 μg/ml prior to GF ingestion to 12.66 ± 2.35 μg/ml (+30.9%, p < 0.001) after 180 minutes (Fig. 2).

Insulin levels rose after meal ingestion, but the IRI 30 minutes after GF intake was lower than that after intake of bread or fried vegetables (Fig. 3a; p = 0.001, p = 0.017). The AUC for IRI after GF intake was lower than that for all other menus (Fig. 3k; p < 0.05). The AUC of IRI/carbohydrate intake was lower when the FV was taken with GF than that after intake of fried vegetables without GF (Fig. 3c; p < 0.001).

Triglyceride level gradually increased 30, 60 and 120 minutes after fried vegetables intake (Fig. 4a), and the triglyceride AUC was lower when GF was eaten prior to fried vegetables (Fig. 4b; p = 0.049). Triglyceride AUC/carbohydrate intake was lower when GF was eaten prior to fried vegetables (Fig. 4c; p < 0.001).
Effect of Grapefruit Intake on Postprandial Plasma Glucose

**Fig. 1a**

**Change of plasma glucose (mg/dl).**

**Fig. 1b**

**AUC of glucose (mg/dl • min)**

**Fig. 1c**

**AUC of glucose / carbohydrate intake**

(a) plasma glucose [mg/dl]; b. area under curve (AUC) of plasma glucose [mg/dl•min];
c. glucose AUC/carbohydrate intake [mg/dl•min/g]. Means ± standard deviation (SD), n = 12.

*** = p < 0.001. GF: grape fruits, FV: fried vegetables.

AUC of each diet compared by Dunnett’s test.
Fig. 2  Vitamin C concentration [µg/ml]

*** = p < 0.001 by paired t-test.
Effect of Grapefruit Intake on Postprandial Plasma Glucose

**Fig. 3a**

**Change of insulin (μU/ml)**

![Graph showing change of insulin](image)

**Fig. 3b**

**AUC of insulin (μU/ml • min)**

![Bar graph showing AUC of insulin](image)

**Fig. 3c**

**AUC of insulin / carbohydrate intake (μU/ml • min/g)**

![Bar graph showing AUC of insulin per carbohydrate intake](image)

**Fig. 3**  
Change of insulin
a. insulin [μU/ml] ; b. area under curve (AUC) of insulin [μU/ml•min] ;
c. insulin AUC/carbohydrate intake [μU/ml•min/g]. Means ± standard deviation (SD), n = 12.
* p < 0.05, ** p < 0.01, *** p < 0.001.
AUC of each diet compared by Dunnett’s test.
GF, grapefruit, FV, fried vegetable.
Effect of Grapefruit Intake on Postprandial Plasma Glucose

**Fig. 4a**

*Change of triglyceride (mg/dl)*

![Graph showing change of triglyceride over time.](image)

**Fig. 4b**

*AUC of triglyceride (mg/dl · min)*

![Graph showing AUC of triglyceride.](image)

**Fig. 4c**

*AUC of triglyceride / carbohydrate intake (mg/dl · min/g)*

![Graph showing AUC of triglyceride per carbohydrate intake.](image)

**Fig. 4** Change of triglyceride

a: triglyceride [mg/dl], b: area under curve (AUC) of triglyceride [mg/dl·min],
c: triglyceride AUC/carbohydrate intake [mg/dl·min/g].

Means ± standard deviation (SD), n = 12. **p < 0.01, ***p < 0.001,
AUC of each diet compared by Wilcoxon test.
GF: grapefruit, FV: fried vegetable.
**Effect of Grapefruit Intake on Postprandial Plasma Glucose**

**Discussion**

Here we found that postprandial blood sugar, IRI and triglyceride concentrations were affected by GF ingestion. In particular, plasma glucose AUC and the ratio of plasma glucose to carbohydrate intake were lower when GF was ingested prior to bread or fried vegetables. The reason that the AUC was divided by carbohydrate intake is to reflect the concept of "glycemic load." "Glycemic load" is the index which divides "glycemic index" exponent with 100 and applies the number of grams weight of carbohydrate included in the food. This index reflects the blood glucose rise after intake of the food more correspondingly than "glycemic index".

These results suggest that some constituent of GF exerts some influences on glycolipid metabolism. We speculate the vitamin C, naringenin (a flavonoid), bergamottin (a furanocoumarin), and dietary fiber in GF improved glycolipid metabolism since previous studies showed the favorable effects of these molecules on glycolipid metabolism. GF contains vitamin C abundantly. Vitamin C is known to reduce oxidation stress and improve insulin resistance, thus exerting a favorable effect on controlling blood glucose.

Naringenin is a known antioxidant and is also reported to have antiviral effect, protect against dimethylnitrosamine-induced liver damage, inhibit tumor growth, accelerate glucose uptake by skeletal muscle, and improve of lipid metabolism.

Naringenin is reported to have a similar molecular structure to estrogen and to bind to estrogen receptor. When orchidectomy rats were fed GF, urinary deoxypyridinoline, which is increased by orchidectomy, decreased to the original level. As orchidectomy changes bone metabolism by increasing bone absorption, these data indicate that bone metabolism may be improved by naringenin supplementation. Naringenin acts like estrogen and increases bone mineral density.

GF has a higher antioxidant activity than other fruits. As there is no correlation between antioxidant activity and the concentration of polyphenol, a major antioxidant, the effect observed following GF intake may be derived from naringenin.

Grapefruit juice contains 7.7 μg/g bergamottin (BG), 8.8 μg/g bergaptol (BT), and 3.7 μg/g 6',7'-dihydroxybergamottin (DHB). At these concentrations, 200 ml of grapefruit juice contains 1.5 mg BG, 1.8 mg BT and 0.75 mg DHB. Bergamottin interferences with CYP3A4 cytochrome P450, and is reported to have antiviral effect, protect against dimethylnitrosamine-induced liver damage, inhibit tumor growth, accelerate glucose uptake by skeletal muscle, and improve of lipid metabolism.

Naringenin is reported to have a similar molecular structure to estrogen and to bind to estrogen receptor. When orchidectomy rats were fed GF, urinary deoxypyridinoline, which is increased by orchidectomy, decreased to the original level. As orchidectomy changes bone metabolism by increasing bone absorption, these data indicate that bone metabolism may be improved by naringenin supplementation. Naringenin acts like estrogen and increases bone mineral density.

**Effect of grapefruit on sugar and lipid metabolism**

In trials using experimental rats fed cholesterol-rich food, lipid metabolism is improved by GF intake. This finding is explained by the anti-oxidative action of GF. Grapefruit oil limited the generation and reduced the activity of glycerol-3-phosphate dehydrogenase (GPDH) by 70% in cell cultures of subcutaneous adipocytes and PPARγ gene expression which suggests that GF may reduce intracellular fat accumulation.

**Effect of citric acid on intracellular glucose**

High sugar intake may increase intracellular glucose concentration. In turn, this may increase glycation (saccharification) stress and interfere with the tricarboxylic acid (TCA) cycle in mitochondria. Excessive glucose in fat cells generates fumaric acid, which may react with cysteine in proteins. This reaction produces S-(2-succinyl) cysteine (2SC) which affects proteins, such as cytoskeleton protein, cytokine, heat shock protein and adiponectin.

In the streptozotocin-induced DM rats, oral administration of citric acid reduced ketone generation and accumulation of N-(carboxymethyl)lysine (CEL) in the crystalline lens, thus preventing the progress of cataract and renal dysfunction. Since citric acid acts at the beginning of the TCA cycle, elevated levels, such as those arising from GF ingestion, may modify the deleterious affects of excessive glucose and reduce glycation stress.

**Effect of grapefruit on the vascular system**

Grapefruit consumption may decrease coronary vascular resistance and mean arterial pressure. Surveys have found fruit, vegetable, potassium and vitamin C consumption lower the risk of hypertension. Guidelines for the management of hypertension (JSH 2009) recommend that a patient’s diet should include vegetables, fruit, fish or fish oil; patients should restrict salt intake, maintain standard weight, undertake moderate aerobic exercise and quit smoking.

In a previous study, we examined the effects of GF juice on arteriosclerosis in genetically modified (ApoE inactivated) mice. ApoE mice were fed a high cholesterol diet for 21 weeks. One group (n = 10) was given GF juice and the control group (n = 10) was given a fructose solution. The development of atherosclerosis in the aortic valve was delayed in the GF group.

The evidence from the present and other studies indicate women who consume less than 200 g fruit per a day. There are gender differences between metabolic responses to dietary fiber.

Fruit consumption does not influence blood sugar levels after meals in insulin-dependent diabetes mellitus (IDDM) patients receiving intensive insulin treatment or non-insulin-dependent diabetes mellitus (NIDDM) patients, who retain insulin secretion capacity. However fruits with abundant sucrose and low fructose, often increase postprandial glucose in IDDM patients receiving conservative insulin therapy and NIDDM patients losing insulin secretion capacity. In these latter cases, patients should not consume excessive quantities of fruit.

The glycemic index (GI) of different fruits is reported as: grape 76 ± 25%, pineapple 70 ± 47%, persimmon 66 ± 37%, mandarin orange 59 ± 24%, banana 58 ± 20%, watermelon 57 ± 45%, melon 55 ± 28%, strawberry 46 ± 18%, GF 44 ± 17%, and apple 41 ± 15%. The GI of rice is 72 and, apart from grape, pineapple and persimmon, fruit has a lower GI value than rice.
that GF ingredients have a composite influence upon glycolipid metabolism. Dietary fiber reduces the absorption rate of sugar and lipid. Naringenin and bergamottin promote insulin function and increase muscle cell glucose uptake. Citric acid may moderate excessive glucose in the TCA cycle. Other components of GF stimulate the central and sympathetic nervous system to produce heat. Naringenin and bergamottin stimulate intercellular signals that promote sugar metabolism. The duration of these affects remain unclear and if the duration were long, the result of 4th day (GF + fried vegetable) would be over-estimated by GF intake for two continuous days. This may be the study limitation and future research will test the duration of these affects.

Insulin controls blood sugar levels, but other insulin-resistant hormones, such as glucagon, adrenalin, adrenocorticotropic hormone (ACTH), cortisol, or growth hormone are also involved. The present study was limited as it was not feasible to measure the effect of diet on other hormones.

**Grapefruit safety**

Grapefruit peel samples were tested for several biocide residues. Orthophenylphenol (OPP) was detected at low levels; diphenyl (DP) was not detected; thiabendazole (TBZ) was found at 1.4 – 2.0 mg/kg; imazalil (IMZ) was found at average densities 0.77 — 1.4 mg/kg. These biocide levels are below the maximum acceptable daily intake set by the Ministry of Health, Labour and Welfare.

**Acknowledgements**

This study was supported by a research grant from Florida Department of Citrus, United States of America. Part of this study was presented at the 11th Meeting of Japanese Society of Anti-Aging Medicine at Kyoto, 2011.

---

**References**

4) Date T: Cohort study on difference in nutrition intake and the risk of cerebral apoplexy. The Journal of the Osaka City Medical Center 32; 141-166: 1983 (in Japanese)